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TUBERCLE BACILLI IN BUTTER:

THEIR OCCURRENCE, VITALITY, AND SIGNIFICANCE.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., January 22, 1908.

Sir: I have the honor to transmit herewith a manuscript entitled "Tubercle Bacilli in Butter: Their Occurrence, Vitality, and Significance," by Dr. E. C. Schroeder and W. E. Cotton, of the Experiment Station of this Bureau.

The article deals with a phase of the tuberculosis question which has hitherto received less attention than it deserves. The previous work of Doctor Schroeder and other scientists of the Bureau, largely in connection with the tuberculous infection of milk, has demonstrated the imperative necessity of eradicating tuberculosis from our dairy herds in order to avoid the dangers incident to the consumption of this universal product of the cow. The present paper brings to light additional menace to the public health through the presence of tubercle bacilli in butter, which is as common an article of food as milk.

Our present knowledge points unmistakably to the fact that a very large amount of butter infected with tubercle bacilli is daily consumed by our people. Furthermore, the experiments described in this work prove that butter is an ideal environment for the preservation of tubercle bacilli. It is shown that these micro-organisms when embedded in ordinary salted butter remain alive and virulent a long time; that after ninety-nine days they show only a doubtful reduction of pathogenic virulence.

In view of the importance of the subject to the general public, I recommend the publication of the article in the circular series of the Bureau.

A. M. Farrington.
Acting Chief of Bureau.

Hon. James Wilson.
Secretary of Agriculture.

TUBERCLE BACILLI IN BUTTER: THEIR OCCURRENCE, VITALITY, AND SIGNIFICANCE.

INTRODUCTORY.

Among the articles of human food usually eaten in a raw state nothing has a wider distribution than butter, and next to milk nothing fills a more important place. It appears on most tables at every meal and is eaten two or three times daily by almost every person. It may be transported long distances, and those who use it are seldom in a position to inform themselves about its origin and preparation and other facts which have a substantial bearing on its freedom from infection. Hence butter must be regarded seriously as a vehicle through which a live, infectious material may be distributed in a way that leads directly to the human stomach and in a way against which the individual has no means to protect himself.

While other infectious substances may be carried in butter, we will confine our attention at present to the tubercle bacilli it may contain, and briefly discuss their occurrence, vitality, and significance.

THE OCCURRENCE OF TUBERCLE BACILLI IN BUTTER.[a]

On standing, or by passage through a centrifuge, milk separates into three distinct layers—cream, skim milk, and sediment. The character, appearance, and relative quantity of cream and skim milk are too well known to require description. The sediment in fresh, pure, normal milk is so small in amount that it commonly escapes observation; it has a chalky, white appearance, and on microscopic examination is seen to be composed mainly of leucocytes and a little epithelium. In experiments made at the Bureau of Animal Industry Experiment Station a number of years ago, and recently repeated, it was found that tubercle bacilli, when they are present in milk, soon disappear from the skim milk and collect in about equal proportion

[a] A somewhat similar though independent investigation to that recorded in this circular is being conducted by Mohler, of the Pathological Division, and Rogers, of the Dairy Division of this Bureau, the butter being made from commercial cream by the usual commercial methods and then subjected to the same treatment in cold storage as obtains in the butter trade.

in the cream and the sediment. This was true whether the cream separated slowly and naturally or whether it was forced to the surface of the milk quickly by the use of a centrifugal machine.

As tubercle bacilli have a higher and cream has a lower specific gravity than milk it does not seem unreasonable to infer that the bacilli should gravitate away from the cream and leave it free from infection as it rises to the surface of infected milk. This inference, however, is erroneous; it would probably be true if cream was a homogeneous substance and not an aggregation of small spheres or globules. The minute bacilli evidently adhere to the relatively large cream globules with a tenacity that can not be broken by the existing difference of specific gravity, even when this is magnified by the application of a centrifugal force strong enough to press the cream into a semisolid mass. From this conduct of tubercle bacilli in milk we may conclude that when they are present in milk they will be present in greater concentration in cream, and cream is the substance from which butter is made.

That tubercle bacilli are frequently present in milk has been demonstrated over and over again. Recently 26 samples of milk were taken directly from the Washington supply, each from a different dealer, and tested at the Experiment Station by guinea-pig inoculation, and 2, or 7.7 per cent, produced generalized, fatal tuberculosis.

Butter made from infected cream contains tubercle bacilli. This statement was verified by making butter from several lots of infected cream and testing its infectiousness through guinea-pig inoculations. The cream was derived in some instances from normal cow's milk that was intentionally soiled with small quantities of feces from cows that were passing tubercle bacilli per rectum, and in other instances from the milk of a tuberculous cow with a diseased udder.

TUBERCLE BACILLI MORE FREQUENT IN BUTTER THAN IN MILK.

Our results, as well as those of other observers, indicate that tubercle bacilli may gain a sufficient concentration in butter to serve for their detection even when it is made from milk in which they are too widely scattered to be found. Herr and Beninde[a] concluded from their investigations that skim milk, buttermilk, cream, butter, and sediment from infected milk contain tubercle bacilli, and that the most intensely infected of these substances are sediment and butter. Among 444 samples of butter tested by themselves and others, 60, or 13.5 per cent, were found to contain tubercle bacilli.

[a] Zietschrift für Hygiene, etc., Vol. 38, p. 180.

Cornet [a] gives a table in his recent work on tuberculosis from which an idea of the relative frequency with which milk and butter are infected can be obtained. The table includes the tests made from 1890 to 1902 by a large number of reliable investigators. Among 1,527 samples of milk, 149, or 9.76 per cent, and among 775 samples of butter, 100, or 12.9 per cent, were found to contain tubercle bacilli. Broërs,[b] of the Netherlands, has shown that 10 per cent of the milk of his country is infected with tubercle bacilli.

The figures here quoted refer to conditions in European countries. The instructive and important feature about them for us is that they justify the conclusion that tubercle bacilli can be found more frequently in butter than in milk. The percentage for milk given by Broërs and that obtained from Cornet's table—10 and 9.76, respectively—are almost identical, and the same is true of the percentages of butter, namely, 13.5 by Herr and Beninde and 12.9 by Cornet. They enable us to say that butter probably contains tubercle bacilli in discoverable numbers 13 times for every 10 times they are sufficiently numerous in milk to be detected.

We have satisfactory reasons for believing that European cattle are more commonly tuberculous than American, and, consequently, that the figures obtained by European investigators regarding the frequency with which tubercle bacilli occur in milk and butter are too high for our country. But even with us the conditions are bad enough, as from 15 to 30 per cent of the cows from which our cities derive their milk supply are affected with tuberculosis. What this enormous number of tuberculous cows in dairy herds means for milk and butter infection may be judged from the facts presented in a bulletin [c] of this Bureau dealing with the danger from tubercle bacilli, not alone in the milk of tuberculous cows, but also in the milk of healthy cows stabled in a tuberculous environment.

Milk to be at all times free from tubercle bacilli must be drawn from cows neither affected with nor exposed to tuberculosis; in other words, it must be obtained under conditions that are not provided in the majority of our dairy herds, and hence tubercle bacilli are frequently present in milk. Since their presence in milk has been shown to mean their occurrence in greater concentration in cream and butter, we may end this portion of our article with the conclusion that butter must be regarded under existing conditions as a common vehicle for the dissemination of tubercle bacilli from cattle in a way that insures the exposure of persons to them.

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[a] Die Tuberkulose, Vienna, 1907, pp. 122, 123.

[b] Zeitschrift für Tuberkulose, etc., Vol. X, No. 3.

[c] Bureau of Animal Industry Bulletin 99.

THE VITALITY OF TUBERCLE BACILLI IN BUTTER.

The precise period of time during which tubercle bacilli remain alive and retain their virulence in butter is a question for the solution of which the available data are exceedingly contradictory. This is well shown by Cornet,[a] who states that Laser could find no live tubercle bacilli in butter after twelve days; that Heim records that all tubercle bacilli eventually die in butter, and that their maximum life in it is thirty days; that Gasperini found a reduction of virulence after thirty days, though the bacilli were still alive after one hundred and twenty days, and that Dawson did not observe a reduction of virulence until after three months and claims to have produced tuberculosis in a guinea pig by inoculating it with butter 8 months old.

The two extremes, twelve days and eight months, are too far apart to be satisfactory; either or both may be wrong; both certainly can not be right. A fairly large difference in the results obtained by different investigators may be reconciled on the assumption that they used different kinds of butter in their tests. Salt has distinct though weak germicidal properties; hence tubercle bacilli in heavily salted butter may live only a short time while in unsalted butter they may live and remain virulent indefinitely. Broërs,[b] whose work on the presence of tubercle bacilli in dairy products seems very reliable, found that they will live three days in milk even when it has undergone changes to make it unfit for use as food, twelve days in buttermilk, and that they remain virulent in butter three weeks.

INOCULATION TESTS WITH GUINEA PIGS.

To determine more definitely how long the bacilli actually do live and retain their virulence, some infected butter was prepared at the Bureau Experiment Station and tested with guinea-pig inoculations.

The milk of a cow affected with udder tuberculosis was drawn in the customary manner and set aside in glass dishes for cream to rise. The dishes had straight sides and were 10½ inches broad and 5½ inches deep. After twenty-four hours the cream was carefully skimmed from the surface of the milk and at once churned in a glass churn with a metal thrasher. When the butter had separated from the cream it was gathered and washed and worked until it was free from buttermilk, and then salted at the rate of 1 ounce of salt to a pound of butter. While the amount of salt in commercial butter varies greatly, this is the proportion most commonly used. The salt

[a] Die Tuberkulose, Vienna, 1907, p. 124.

[b] Zeitschrift für Tuberkulose, Vol. X, No. 3.

was worked into and distributed throughout the entire mass as evenly as possible, and the finished product was placed in a glass dish with a ground-glass cover and kept without ice in a cellar in which the temperature remained fairly constant at 60° F.

From time to time guinea pigs were inoculated with portions of the butter; the amount received by each guinea pig, with the exception of Nos. 9683 and 9684, was 1 gram (15½ grains), injected, slightly warmed, from a syringe into the abdominal cavity. The two exceptions received only one-half gram each, as an insufficient supply of butter remained at the time they were inoculated to give them a full dose.

Table 1 gives the numbers of the guinea pigs, the dates of inoculation and death, the age of the butter at the time of inoculation, the number of days that elapsed between the inoculation and the death of each guinea pig, and the condition of the guinea pigs on post-mortem examination.

TABLE 1.—*Results of guinea-pig inoculations with infected butter of various ages.*

No. of guinea pig.	Date of injection.	Age of butter, in days.[a]	Date of death.	Number of days from injection to death.	Autopsy records.
	1907.		1907.		
9250	May 9	1	July 5	57	Generalized tuberculosis.
9251	do	1	July 10	62	Do.
9252	May 10	2	July 5	56	Do.
9253	do	2	July 14	65	Do.
9254	May 15	1	July 5	51	Do.
9255	do	1	July 25	71	Do.
9256	May 16	2	May 23	7	Peritonitis.
9257	do	2	Aug. 15	91	Generalized tuberculosis.
9258	May 17	3	July 2	46	Do.
9259	do	3	July 19	63	Do.
9260	May 18	4	June 30	43	Do.
9261	do	4	July 15	58	Do.
9274	May 20	6	July 9	50	Do.
9275	do	6	July 17	58	Do.
9276	May 21	7	June 3	13	Inflammation of bowels and tuberculosis.
9277	do	7	July 29	69	Generalized tuberculosis.
9306	May 22	8	July 8	47	Do.
9307	do	8	July 22	61	Do.
9308	May 23	9	July 25	63	Do.
9309	do	9	July 9	47	Do.
9316	May 24	10	July 25	62	Do.
9317	do	10	Sept. 25	124	Do.
9336	May 25	11	July 8	44	Do.
9337	do	11	Aug. 12	79	Do.
9350	May 27	13	July 14	48	Do.
9351	do	13	Aug. 28	93	Do.
9352	May 28	14	June 5	8	Pneumonia.
9353	do	14	July 18	51	Generalized tuberculosis.
9354	May 29	15	June 4	6	Pneumonia.
9355	do	15	July 9	41	Generalized tuberculosis.
9356	June 1	18	June 30	29	Do.
9357	do	18	July 26	56	Do.
9358	June 4	21	Aug. 1	58	Do.
9359	do	21	Aug. 4	61	Do.
9402	June 7	24	Aug. 1	55	Do.
9403	do	24	Sept. 16	101	Do.
9416	June 10	27	July 25	45	Do.

[a] Guinea pigs 9250 to 9253, inclusive, were inoculated with butter made on May 8, 1907; the remainder with butter made on May 14, 1907.

TABLE 1.—*Results of guinea-pig inoculations with infected butter of various ages*—Continued.

No. of guinea pig.	Date of injection.	Age of butter, in days.	Date of death.	Number of days from injection to death.	Autopsy records.
	1907.		1907.		
9417	June 10	27	Oct. 1	113	Generalized tuberculosis.
9430	June 13	30	Aug. 16	64	Do.
9431	----do---	30	Sept. 11	90	Do.
9432	June 15	32	July 5	20	Pneumonia and tuberculosis.
9433	----do---	32	Aug. 14	60	Generalized tuberculosis.
9549	July 2	49	Aug. 9	38	Do.
9550	----do---	49	Aug. 19	48	Do.
9647	Aug. 1	79	Oct. 21	81	Do.
9648	----do---	79	Oct. 26	86	Do.
9677	Aug. 9	87	Oct. 21	73	Do.
9678	----do---	87	Nov. 2	85	Do.
9683	Aug. 21	99	Nov. 4	75	Do.
9684 [a]	----do---	99	Nov. 11	82	Do.

[a] All the guinea pigs died naturally with the exception of this one, which was killed for autopsy.

The total number of guinea pigs inoculated was 50. Of these, 5 died prematurely—1 affected with peritonitis, 1 with inflammation of the bowels, and 3 with pneumonia. Two of the 5 showed lesions of tuberculosis, and 3 died too soon after inoculation for tuberculosis to have developed sufficiently for detection. One guinea pig (No. 9684) was intentionally killed, and though it was affected with generalized tuberculosis its condition indicated that it would have survived ten days or two weeks longer had it been permitted to live. This leaves 44 guinea pigs that died of generalized tuberculosis, uncomplicated with other affections, due to virulent tubercle bacilli contained in the butter with which they were inoculated. An examination of the time that elapsed between inoculation and death of the 44 guinea pigs gives less evidence to show that tubercle bacilli in butter lose much of their virulence in the course of ninety-nine days than positive evidence of a great variation in the susceptibility of the guinea pigs to tuberculous infection. For example, one of the two guinea pigs inoculated with butter 10 days old died after sixty-two days and the other lived twice as long, one hundred and twenty-four days. Of the two guinea pigs inoculated with butter 27 days old one died in forty-five days and the other lived one hundred and thirteen days. Of the two guinea pigs inoculated with butter 49 days old one lived only thirty-eight and the other only forty-eight days; that is, both succumbed in less time than any of the four guinea pigs inoculated with butter only one day old. The butter 79, 87, and 99 days old failed to kill any of the six guinea pigs inoculated with it in less than seventy-three days. This is a little longer than the

average for the butter that had been kept 49 days or less, but the guinea pigs inoculated with butter 99 days old received only a half dose, and one of them succumbed in seventy-five days.

We may safely conclude from the guinea-pig inoculations that tubercle bacilli show no appreciable attenuation in ordinary salted butter in forty-nine days, and that they are still highly virulent after ninety-nine days, or more than three months.[a] This conclusion is fairly compatible with the results obtained by Dawson, who found that no attenuation occurred until after three months, and with those of Gasperini, who found that bacilli were still alive after four months, though he observed some attenuation after thirty days.

SIGNIFICANCE OF TUBERCLE BACILLI IN BUTTER.

The relation of tubercle bacilli in butter to the public health is a more involved question than their simple presence and long-continued life and virulence.

Less than ten years ago, previous to the end of the last century, tubercle bacilli were grouped for all practical purposes in two classes, mammalian and avian, or those which affected man and other mammals and those which affected birds. No one doubted openly that bacilli from cattle, in meat and dairy products, were as injurious for man as those derived from persons. Pulmonary tuberculosis, or consumption of the lungs, was then, as now, the commonest form in which the disease manifested itself, and this was explained by the assumption that the bacilli entered the body more frequently with the breath than in any other way, and that the greatest danger of infection was through dried and pulverized tuberculous material that floated in the air as a fine dust.

The beginning of the present century brought with it a change of views. Attention was called to the fact that the inhalation theory to account for the frequent presence of tuberculosis in the pulmonary tissues had not been proven, and that living tubercle bacilli in dust were difficult to find or could not be found at all. The infectiousness of bacilli from animals for man was questioned, and the investigation of tuberculosis generally was given a fresh impetus through which many new facts and theories came to light.

As tubercle bacilli in butter are derived from bovine sources and usually enter the body only in a moist state, to understand the true significance they have for public health we must give some attention to the infectiousness of tubercle bacilli from bovine sources for man, and to the ways in which tubercle bacilli enter the bodies of those who become affected with tuberculosis.

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[a] See note at end of circular.

Since Theobald Smith [a] published his studies on different varieties of tubercle bacilli, the evidence in favor of two distinct types virulent for mammals—the one found more commonly in bovine and the other in human lesions—has grown stronger. But different varieties or types do not necessarily mean different species or even subspecies. As Smith himself stated, "varieties have been found among nearly all of those specific forms of pathogenic bacteria which have received a considerable amount of attention." [b] The term "varieties" is here clearly used to designate differences of a kind to be expected among the individuals of a large and widespread species, such differences as we know occur among higher organisms than bacteria with a wide geographic distribution. There is a distinct parallelism between a wide geographic distribution of higher plants and animals and the number and kinds of hosts a pathogenic bacterium may infect; hence there is no reason why the tubercle bacillus, which has received more attention and which affects more species of animals and more individuals than any other bacterium, should not have been found to include many different types, the extremes of which would leave us in doubt as to their specific classification if they were not connected by a chain of transition forms.

Mohler and Washburn,[c] after a comparison of many tubercle bacilli from different sources and a careful search of the literature, concluded that the more the subject is studied the more numerous the instances become in which bacilli of special types are found occurring naturally in animals far removed from the species which may be supposed to be their natural host. They obtained cultures of tubercle bacilli from human lesions that were morphologically and biologically bovine types, and in their summary of the investigations of others show that bovine types have frequently been obtained from man and human types from cattle.

Fibiger and Jensen,[d] who likewise obtained typical bovine bacilli, virulent for cattle, from human lesions, recall that the Imperial German Health Office examined 39 cases of primary tuberculosis of the intestine and mesenteric glands and found that 13 among them were caused by bacilli of the bovine type.

Gorter,[e] after a careful study of tubercle bacilli from human and bovine lesions, found 7 among 21 cultures from human sputum which

[a] Twelfth and Thirteenth Annual Reports, Bureau of Animal Industry, 1895 and 1896. Journal of Experimental Medicine, Vol. 3, New York, 1898.

[b] Twelfth and Thirteenth Annual Reports, Bureau of Animal Industry, 1895 and 1896, p. 149.

[c] Bureau of Animal Industry Bulletin 96.

[d] Berliner Klinische Wochenschrift, Nos. 4 and 5, 1907.

[e] Zeitschrift für Tuberkulose, Vol. XI, No. 3, 1907. Also Inter. Centralb. für die ges. Tuber. Fors., Vol. II, No. 1, 1907.

he regards as identical with the transition forms between human and bovine bacilli which he says are described by Rabinowitsch. He concludes that human and bovine bacilli are not different varieties, and that the conversion of the one type into the other actually occurs.

Sargo and Suess[a] showed that mutations occur in human tubercle bacilli and other types, which speak against grouping tubercle bacilli from animals of different species as special varieties.

Von Behring,[b] who ranks as one of the most widely recognized authorities on tuberculosis, found cultures of tubercle bacilli isolated from man of low virulence for cattle, and others of higher virulence for them than many cultures of bovine origin. He declares himself as opposed to the view that bovine tubercle bacilli may be harmless for man, and calls attention to the fact that they generally have a higher grade of virulence than human bacilli and are therefore to be regarded as more dangerous.

The British Royal Commission on Human and Animal Tuberculosis[c] concluded from its investigations that cow's milk containing bovine tubercle bacilli is clearly a cause of tuberculosis, and of fatal tuberculosis in man, and that a very large portion of tuberculosis contracted by ingestion is due to tubercle bacilli of bovine origin.

It does not seem necessary to add to this evidence to prove that the various existing types of tubercle bacilli are simply mutation forms of one specific organism. The presence of transition forms between human and bovine types; the occurrence of pure bovine types in human lesions and of human types in bovine lesions; the occurrence of bacilli highly virulent for cattle in human lesions; the generally greater virulence of bovine types for all species of animals, and the virulence, and greater virulence, of bovine types for anthropoid apes and monkeys,[d] or the animals in the zoological scale most nearly related to man, are facts that support the conclusion that bovine tubercle bacilli in butter and other dairy products are a source of great danger to public health.

THE GREATER FREQUENCY OF LUNG TUBERCULOSIS.

It is a fact that tuberculosis is more commonly an affection of the lung than of other portions of the body. The explanation for this, which was long regarded as satisfactory and is still accepted by many, rests on the assumption that the most important source of tuberculous infection is finely pulverized tuberculous material, sus-

[a] Centralb. für Bacteriologie, etc., Vol. XLIII, Part I, p. 422–529.

[b] Berliner Tierärz. Wochens., No. 47, 1902.

[c] Jour. Royal Institute of Public Health, Vol. XV, No. 3, 1907.

[d] Report of the British Royal Commission in the British Jour., No. 2430, 1907; Bureau of Animal Industry Bull. 52, 1905.

pended in the air as dust, and the direct exposure of the lung to this dust through the process of respiration. If this so-called inhalation theory is true, and, as many of those who maintain it assert, tubercle bacilli can not pass through the uninjured wall of the digestive tract and reach the organs remote to it without leaving evidences of their passage, then tubercle bacilli in butter and other articles of food eaten by adults have no important significance for public health. Therefore to prove that tubercle bacilli in butter and food products of all kinds are dangerous we must give some thought to the mode of infection, or the portal through which the bacilli enter the body.

How strongly the inhalation theory was intrenched in the minds of medical men until quite recently is well expressed by Aufrecht[a] in the statement that considerable courage was required only a few years ago to characterize the theory as an unwarranted hypothesis for the wide belief of which no satisfactory evidence had been supplied. He, in 1900,[a] and Baumgarten,[b] in 1901, pointed out that it had not been proven to be the exclusive or even the most important mode of infection with tuberculosis. In 1902 followed the experiments of Nicolas and Descos,[c] confirmed by those of Ravenel[d] in 1903, which proved that tubercle bacilli introduced into the healthy intestinal canal of animals rapidly passed through the uninjured mucosa and appeared in the great thoracic duct on their way to the venous circulation. Nocard and his pupils, Desoubry and Porcher,[e] had earlier shown that the passage of bacteria through the intestinal wall and their transference to the blood was possible. Chauveau,[f] in view of the constantly accumulating evidence that pulmonary tuberculosis in man and animals arises from infection through the intestine, calls attention to his investigations from 1868 to 1874, in which pulmonary tuberculosis was brought about by the ingestion of tuberculous material without the production of pathological conditions in the digestive tract.

This earlier work was followed rapidly by other investigations, which proved more and more conclusively that the introduction of tubercle bacilli into the body with food may lead directly to the development of pulmonary tuberculosis, without lesions in the alimentary canal and without intermediate lesions of disease between the digestive and respiratory organs. The most important investigations are prob-

[a] Berliner Klinisch Wochens., No. 27, 1907.

[b] Wiener Med. Wochens., Vol. 51, No. 44.

[c] Jour. Phys. et de Path. Gén., Vol. IV, 1902.

[d] Jour. Med. Resea., Vol. X, pp. 460–462.

[e] Comp. Rend. Soc. de Biologie, Vol. XLVII, 1895.

[f] Experiment Station Record, U. S. Dept. of Agri., Vol. XIX, No. 2, 1907. (Comp. Rend. Acad. Sci., No. 15, Paris, 1907.)

ably those of Calmette and his associates, recently published in book form.[a]

These investigators claim, and present good evidence in support of their claim, that dust particles that enter the lung never penetrate deeper than the first branches of the bronchi; that tuberculosis is constantly a disease of which the infection enters through the intestine; that tubercle bacilli may penetrate the intestinal wall without causing lesions; that the bacilli may pass through the mesenteric glands without causing lesions; that the bacilli frequently cause primary lesions in the mesenteric glands of young experiment animals, but commonly pass through these glands of adult animals and cause primary pulmonary tuberculosis; that tuberculous processes in the lung never begin in the bronchi or alveoli, but constantly in the capillaries, especially in the finest capillary network of the subpleural tissue, etc.

Relative to this localization of the earliest stages of pulmonary tuberculosis, Aufrecht[b] says "The fact is that the initial changes in the apices of the lung, as I have convinced myself by repeated anatomical examinations, do not spread from the terminal branches of the bronchi." He further says that he has "proven the cheesy tubercle in the lung to be associated not with the final branches of the air tubes, but with the terminal capillaries of the pulmonary arteries." While he is not a special advocate of the intestinal way as the sole mode of infection, he ends his article here referred to with these words: "The inhalation theory for lung tuberculosis is no longer tenable." Köhler,[c] who reviews Aufrecht's work, justly remarks that it deserves a wide recognition, as it supplies important arguments for a thorough revision of the older views about the development of pulmonary tuberculosis.

Fibiger and Jensen[d] conclude from their own investigations and a critical analysis of the reports from numerous widely separated hospitals that the former doctrine, which taught that primary intestinal turberculosis is a rare disease, can no longer be held as valid. Among 289 children from 1 to 15 years old who had succumbed to various diseases, 44, or over 15 per cent, were found on autopsy to be affected with primary intestinal tuberculosis. These investigators

[a] Recherches expérimentales sur la Tuberculose, effectuées à l'institut Pasteur de Lille, par Calmette et Guérin, P. Vansteenberghe, M. Breton, Grysez, Sonneville et Georges Petit, Paris, 1907. Reviewed in the monthly publication of the International Antituberculosis Association, Tuberculosis, Vol. VI, No. 5, 1907, pp. 256–259. Also in Zeitschrift für Tuberkulose, Vol. XI, No. 2, 1907, pp. 163–166.

[b] Berliner Klinische Wochens., No. 27, 1907.

[c] Intren. Centralb. für die gesam. Tuber. Forsch., Vol. II, No. 1, 1907.

[d] Berliner Klinische Wochens., Nos. 4 and 5, 1907.

say that we must, without doubt, return to our former view and regard the ingestion of raw milk as an important cause of primary intestinal tuberculosis during childhood. This view is in perfect harmony with Calmette's experiments, which proved that primary intestinal tuberculosis is of more common occurrence, with infection that enters the body through the alimentary canal, in youth than in adult life.

Orth [a] makes the statement that even with localized tuberculosis in the lymph glands and the lung we can not exclude the intestine as the portal of entry for the tubercle bacillus. At the international conference on tuberculosis, held in Vienna during September, 1907, he said that tubercle bacilli can enter the body from the intestinal canal, which might itself, however, remain completely unaffected, but that from the prophylactic point of view the channel of infection was of only secondary importance, as the object to be aimed at was the destruction of all sources from which infection might take place. As sources of infection he named milk and butter from tuberculous cows and sputum from tuberculous individuals, and bovine tuberculosis was characterized by him as undoubtedly infectious for human beings.[b]

Klebs [c] has convinced himself that tuberculosis is a disease of the lymphatic system and may remain such until the end of life, and that infection occurs through the intestines, most frequently with bacilli contained in cow's milk. He claims to have established this as a fact with experiments made at Berne, and published in Virchow's Archives in the early seventies of last century. He says that he has found no reason to change his views, and calls attention to the conclusive manner in which they have been proven by the unimpeachable experiments of Orth, Von Behring, and Calmette.

Gorter [d] adds his testimony to show that the intestinal mode of infection is not rare, and Bongert [e] showed with rats, as was shown by this Bureau [f] with hogs and cattle, that the injection of pure cultures of tubercle bacilli into portions of the body as remote as possible to the thorax caused pulmonary tuberculosis, and that without intermediate lesions to connect the location of the disease in the lungs with the portal at which the infecting bacilli were introduced.

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[a] Berliner Klinische Wochens., No. 8, 1907.

[b] Editorial in New York Medical Record, Vol. 72, No. 22, 1907, p. 905.

[c] Deutsch Medic. Wochens. No. 15, 1907.

[d] Zeitschrift für Tuberkulose, Vol. XI, No. 3, 1907. Also Intern. Centralb. für ger. Tuber. Forsch., Vol. II, No. 1, 1907.

[e] Tierärz. Wochens., Vol. XV, No. 29, 1907.

[f] Bureau of Animal Industry Bulletin 93, 1906.

Baumgarten [a] concluded after experimental studies and a review of the literature that for practical, prophylactic purposes we must consider not only the inhalation theory and ingestion as modes of infection, but all possible ways in which tubercle bacilli may enter the body.

It is not intended to give a complete summary of all the investigations that have supplied evidence to support the fact that tubercle bacilli can and do penetrate the wall of the digestive tract without affecting it and pass to the lung and there cause lesions. We have amply shown that the intestinal mode of infection for pulmonary and other forms of tuberculosis, unlike the inhalation of tubercle bacilli directly into the lung tissue, is not merely a theory, but a well-established truth, which has forced its way to recognition in the face of considerable opposition. Hence we may assert that the frequency with which tuberculosis is a pulmonary disease can not be used as an argument to encourage an undervaluation of tubercle bacilli in butter; on the contrary, the mode of infection with tuberculosis, the certainty with which tubercle bacilli may enter one portion of the body and leave it unaffected and cause disease in other portions, condemns butter infected with tubercle bacilli as a serious menace to public health.

RELATIVE VIRULENCE OF TUBERCLE BACILLI IN VARIOUS SUBSTANCES.

A few words are required about the relative virulence of tubercle bacilli (1) in moist, opaque substances like milk, cream, butter, and cheese; (2) in dry dust from tuberculous material; (3) in translucent substances like sputum, and (4) in transparent substances like the infectious spray of droplets that may escape from the mouths of tuberculous subjects during more or less violent expiratory efforts.

Cornet [b] is probably the strongest advocate of the dust-inhalation hypothesis. According to his views, dried, pulverized tuberculous sputum is the most important factor for the dissemination of tubercle bacilli and the transmission of tuberculosis from person to person, notwithstanding that he himself calls attention to the rapidity with which the bacilli die upon exposure to light and drying, and to the difficulty with which a tough, sticky substance like sputum is pulverized; also the fact that only a small fraction of a mass of sputum can reach a sufficiently fine state of pulverization to float in the air, or that fine state which he believes necessary for its direct introduction into the finest branches of the bronchial tubes.

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[a] Inter. Centralb. für die ges. Tuber. Forsch., Vol. II, No. 1, 1907.

[b] Die Tuberkulose, Vienna, 1907, pp. 101–117.

The contrast between the life of tubercle bacilli from the same source in butter, 99 days or more, and in material that may become pulverized sufficiently under favorable circumstances to float in the air, is very impressive. It must be borne in mind that material sufficiently pulverized to float in the atmosphere of rooms is in a very fine state of subdivision, and hence offers an enormous surface, relative to its mass, for light to act upon and exert its germicidal influence. In the open, on streets and highways, where currents of air are stronger, particles of sputum may be blown about long before they can be suspended in the air in a way that will lead to their introduction into the mouth and nasal chambers, and their movement from place to place will insure their sufficient exposure to direct light to bring about their entire sterilization before they are so finely pulverized that they can be carried far into the body by respiratory processes.

Flügge and his associates[a] first showed that Cornet's dust-inhalation hypothesis was faulty, and proved that even in the environment of tuberculous persons it was difficult to obtain dust capable of floating in the air that contained living, virulent tubercle bacilli. For dust they substituted the so-called droplet theory. While it is certain that droplets containing virulent tubercle bacilli are expelled from the mouths of tuberculous persons during coughing and speaking in the form of a fine spray, they can have no great importance during their suspension in the air beyond the immediate environment of such persons, and hence can not account for the great frequency of tuberculosis as a disease of man and animals.

The droplets are either so heavy that they settle at once and attach themselves to various articles from which they can not be removed without considerable friction, or so small that the light can act on the contained bacilli from all sides and deprive them of their infectiousness in a very short time. The large droplets are a source of danger when articles of food are handled by or exposed to persons affected with tuberculosis, and because of this danger it is hoped that the time is not distant when stringent regulations will make it impossible for persons known to be tuberculous to come in contact in any way with food that is eaten by others, especially with food that is presented for sale in public markets. Bread, cake, milk, butter, fruit, etc., which are eaten in the condition in which they are purchased or received from the dealer may be a serious tuberculous danger when they are talked and coughed over by persons affected with consumption or any form of open tuberculosis.

[a] Zeitschrift für Hygiene, Vol. 38. See also editorial in the Journal of the American Medical Association, October 12, 1901.

What we have said about sputum, as we stated in a former publication, must not be taken or used as an argument in favor of indiscriminate and unrestricted spitting. Although dried and pulverized tuberculous sputum holds no terrors that we can discover, there are no reasons to doubt that fresh, moist tuberculous sputum is a prime agent for the transmission of tuberculosis from person to person.

When sputum is deposited promiscuously on streets and in public places it may easily collect in the form of smears on shoes, trousers, and, more commonly and in larger amounts, on the skirts of women, and be carried into dwellings, where small masses are transferred to floors and furniture. This matter is not properly relevant to our subject, and therefore can not be discussed here with the care its importance merits. But no great imagination is needed to picture how sputum collected on the apparel of persons in the streets and elsewhere may indirectly infect articles of food, and how, in various and numerous ways, it may be a constant tuberculous menace in otherwise wholesome and sanitary habitations. Little children are first of all and most seriously exposed, because they are of low stature and the portion of the clothing most commonly soiled is that nearest the floor, which may have become contaminated by means of soiled shoes, skirts, etc. They spend much time playing on the floor and they frequently put their hands, soiled or clean, into their mouths, and are apt to eat articles that have been in contact with the floor without a thought about dirt or infection.

Unguarded spitting is dangerous no matter by whom it is practiced, because it is not only the sputa of those who are consciously affected with tuberculosis that contain tubercle bacilli, but also that of persons who are apparently well, but unconsciously affected. The insidious character of the disease insures that persons of the latter kind will be fairly numerous in the places where free spitting can do the most harm.

THE EFFECT OF SUNLIGHT ON TUBERCLE BACILLI.

The rapidity with which tubercle bacilli die on exposure to light is shown by a series of guinea-pig inoculations made at the Experiment Station with tuberculous pus obtained from the udder of the same cow that supplied the milk from which the butter, used in the butter guinea-pig inoculations, was prepared.

After May 8 and 14, the dates on which the two lots of butter used were prepared, the tuberculous disease of the cow's udder progressed very rapidly until a large tumor had formed which broke open and discharged a thick, glistening, pale yellow pus. Some of this pus was collected on September 24 and used as follows: A portion was spread smoothly in a translucent layer on a sheet of glass and exposed to

direct sunlight; a second portion was similarly spread on glass and exposed to ordinary room light, but at no time to the direct rays of the sun, and a third portion was put on a glass plate in thick, heavy masses, fully as large as the largest masses of sputum ejected by tuberculous persons, and exposed to direct sunlight. With material from the three sheets of glass guinea pigs were inoculated from time to time, as is shown in the following tables. The inoculations were all subcutaneous and the dose of material in each instance was 1 milligram (about $\frac{1}{65}$ grain).

The thin layers of tuberculous pus on the plates dried rapidly, the thick masses slowly. The material was more nearly opaque than sputum, but not so adhesive nor so elastic or tenacious; it contained innumerable tubercle bacilli. Compared with sputum it was easily pulverized, but for this purpose it was necessary to scrape it from the plates with a sharp instrument and to rub it for some time between two hard surfaces. The thick material was more easily removed from the plates, but not so easily pulverized; it had a tendency to break into tough, heavy scales, which, when ground, at first formed a coarse, heavy powder, of which the individual granules were spherical and difficult to crush.

The results of the guinea-pig inoculations with the three different kinds of tuberculous material are shown in Tables 2, 3, and 4.

TABLE 2.—*Results of guinea-pig inoculations with tuberculous material exposed in thin layer to direct sunlight.*

No. of guinea pig.	Number of hours material was exposed.	Date of inoculation.	Date of death.	Days from inoculation to death.	Result.
		1907.	1907.		
9021	1	Sept. 24	Oct. 30	36	No tuberculosis.[a]
9022	1	----do----	Oct. 28	34	Do.
9023	2	----do----	----do----	35	Do.
9024	2	----do----	Nov. 5	42	Do.
9025	3	----do----	Oct. 31	37	Do.
9026	3	----do----	Sept. 27	3	Do.
9027	4	----do----	Oct. 30	36	Do.
9028	4	----do----	----do----	36	Do.
9029	5	----do----	----do----	36	Do.
9030	5	----do----	Nov. 1	38	Do.
9035	6	Sept. 25	Nov. 2	38	Do.
9036	6	----do----	Nov. 1	37	Do.
9039	7	----do----	----do----	37	Do.
9040	7	----do----	Oct. 28	33	Do.
9041	8	----do----	Oct. 29	34	Do.
9042	8	----do----	Oct. 28	33	Do.
9045	9	----do----	Oct. 30	35	Do.
9046	9	----do----	Oct. 29	34	Do.
9047	10	----do----	Nov. 4	40	Do.
9048	10	----do----	Oct. 28	33	Do.

[a] The guinea pigs all died prematurely affected with inflammation of the bowels, due to unknown causes. With one exception, No. 9026, they lived long enough after inoculation for lesions of tuberculosis to develop.

TABLE 3.—*Results of guinea-pig inoculations with tuberculous material exposed in clumps to direct sunlight.*

No. of guinea pig.	Number of hours material was exposed.	Date of inoculation.	Date of death.	Days from inoculation to death.	Result.
		1907.	1907.		
9931	5	Sept. 25	Nov. 8	44	No tuberculosis.[a]
9932	5	---do---	Nov. 4	40	Do.
9949	11	Sept. 26	---do---	39	Do.
9950	11	---do---	---do---	39	Do.
9977	17	Sept. 27	---do---	38	Do.
9978	17	---do---	---do---	38	Do.
0045	22	Oct. 1	---do---	34	Do.
0046	22	---do---	Nov. 8	38	Do.

[a] The guinea pigs all died prematurely affected with inflammation of the bowels, due to unknown causes. They lived long enough after inoculation for lesions of tuberculosis to develop.

TABLE 4.—*Results of guinea-pig inoculations with tuberculous material exposed in thin layer to ordinary room light.*

No. of guinea pig.	Number of days material was exposed.	Date of inoculation.	Date of death.	Days from inoculation to death.	Result.
		1907.	1907.		
9933	1	Sept. 25	Nov. 2	38	Generalized tuberculosis.[a]
9934	1	---do---	Nov. 13	49	Do.
9951	2	Sept. 26	Nov. 4	39	Do.
9952	2	---do---	---do---	39	Do.
9979	3	Sept. 27	Nov. 6	40	Do.
9980	3	---do---	---do---	40	Do.
1	4	Sept. 28	---do---	39	Do.
2	4	---do---	Nov. 2	35	Do.
11	6	Sept. 30	Nov. 11	42	Do.
12	6	---do---	Oct. 31	31	Do.
49	7	Oct. 1	Nov. 6	36	Do.
50	7	---do---	Nov. 2	32	Do.
69	8	Oct. 2	Oct. 31	29	Do.
70	8	---do---	Nov. 18	47	Do.
93	10	Oct. 4	---do---	45	Do.
94	10	---do---	Nov. 4	31	No lesions of tuberculosis.

[a] The guinea pigs, with the exception of Nos. 70 and 93, which were killed for autopsy, were all affected with inflammation of the bowels due to unknown causes.

Unfortunately most of the guinea pigs used in this experiment died prematurely from other causes than tuberculosis. All but one, however, lived long enough after inoculation for even very attenuated tubercle bacilli to have produced unmistakable, well-marked lesions of tuberculosis. The cause of death was invariably inflammation of the bowels, which also attacked a number of other guinea pigs confined in the same detached house. The outbreak of disease was probably due to errors in feeding for which a newly appointed employee, who attended the animals in the house in question, was responsible. This employee, contrary to instructions which he misunderstood, failed to feed the animals a sufficient amount of fresh vegetable matter. The whole investigation is being repeated and the results will be published at a future date.

The first table shows that tubercle bacilli in thin layers of pus, exposed to direct sunlight, die in less than one hour; the second that the same is true with tubercle bacilli in thick clumps of pus exposed less than five hours. How much more quickly the bacilli die than the given number of hours is not known, because it was assumed from the various investigations on the germicidal power of sunlight for tubercle bacilli with which we are acquainted that the bacilli would still be alive in respectively one and five hours. In our new series of experiments tests will be made for shorter periods of time.

The third table shows that tubercle bacilli in thin layers of pus exposed to ordinary room light may be alive after ten days, and it also shows, since the same kind of pus was used in all cases, that the plates exposed to sunlight actually contained tubercle bacilli which were alive and virulent before the rays of the sun killed them.

SUMMARY.

(1) The conduct of tubercle bacilli in milk is to move both upward with the cream and downward with the sediment and thus, in both directions, away from the intermediate layer of skim milk. The downward movement is due to their high specific gravity and the upward movement to the tenacity with which they adhere to the comparatively large cream globules. Hence when cream is separated from infected milk it will contain, volume for volume, more tubercle bacilli than the milk.

(2) The frequency with which tubercle bacilli occur in sediment from milk is a fair measure of the frequency with which they occur in cream. What this means for the infection of commercial cream may be judged from the following paragraph quoted verbatim from the last Annual Report of the Secretary of Agriculture:[a]

> The examination of sediment taken from cream separators of public creameries throughout the country has demonstrated the presence of tubercle bacilli in about one-fourth of the samples.

(3) When butter is prepared from infected cream tubercle bacilli are transferred to it in such numbers that they will be present in greater concentration than in the milk from which the cream was derived; hence, measure for measure, infected butter is a greater tuberculous danger than infected milk.

(4) Tubercle bacilli embedded in ordinary salted butter remain alive and virulent a long time; after ninety-nine days they show only a doubtful reduction of pathogenic virulence.

(5) Butter seemingly contains nothing excepting salt that acts against the life and virulence of tubercle bacilli. The germicidal

[a] Report of the Secretary of Agriculture, Washington, D. C., 1907, p. 30.

value of salt, especially in the proportion in which it is used in commercial butter, is very low. Besides, the distribution of salt in butter is not homogeneous, and hence tubercle bacilli may be so embedded in butter that they are not exposed to the salt it contains.

(6) Sunlight is the most potent, natural agent for the sterilization of tubercle bacilli; it kills them in less than one hour when they are exposed to the direct rays of the sun in translucent layers of infectious pus, and in less than five hours when they are exposed in thick, opaque masses of such pus. Weinzirl[a] asserts that tubercle bacilli, as well as other nonsporulating pathogenic bacteria, are destroyed in from two to ten minutes by direct sunlight, and Koch,[b] Jousett,[c] Flügge,[d] Heymann,[e] Di Donna,[f] Cadéac,[g] and others earlier called attention to the rapidity with which tubercle bacilli are destroyed by desiccation and exposure to light. Hence we may conclude that the conditions by which tubercle bacilli are surrounded in butter, the moist opaque character of which shields them against the germicidal action of light and drying, are ideal for their long preservation. As a matter of fact it is difficult to imagine a better environment for the conservation of the life and virulence of tubercle bacilli not actively associated with tuberculous lesions than butter affords.

(7) Unimpeachable evidence proves conclusively that tubercle bacilli of the bovine type, from bovine sources, must be classed as highly infectious for man; hence, tubercle bacilli in butter can not be ignored because they are usually derived from bovine sources.

(8) Since tubercle bacilli of the bovine type are certainly more virulent than those of the human type for all species of animals with which comparative tests have been made, it seems reasonable to ask, Why should they be regarded as less virulent for man?

(9) Tubercle bacilli of the bovine type are more frequently associated with the tuberculous lesions of children than with those of adults. Does this mean that children are oftener affected with tuberculosis from bovine sources than adults, or does it mean that mutations, shown to occur among tubercle bacilli, have had more time to pass through a complete transition from the bovine to the human type in tuberculous adults than in tuberculous children? This question is

[a] Dept. Agri. Expt. Sta. Rec., Vol. XIX, No. 3, 1907, p. 280. (Jour. Infect. Diseases, May, Sup. 3, pp. 128 to 153.)

[b] Cornet, Die Tuberkulose, Vienna, 1907, p. 41.

[c] Wiener Med. Wochens., 1901, No. 28, p. 1366.

[d] Zeitschrift für Hygiene, Vol. 38.

[e] Editorial, Jour. Amer. Med. Asso., Oct. 12, 1901.

[f] Centralb. für Bact. und Parasitenk., Vol. XLII, No. 7.

[g] Le Bulletin Médical, Sept. 5, 1906.

of special interest in connection with Von Behring's view—that tuberculosis at whatever age it occurs and wherever the lesions are located, arises from latent tubercle bacilli that entered the body through the intestinal canal during childhood.

The authority of Von Behring in the field of tuberculosis is so great that we could not afford to discard his view lightly even if it lacked the abundant support other investigators have given it. If it is true, we certainly have good reasons to believe that the mutations, which quite a number of investigators have recorded as occurring among tubercle bacilli, have had ample time in the lesions of adults to result in a complete adaptive transition from the bovine to the human type of bacillus. Tuberculosis is undoubtedly contracted from two great sources, namely, human tuberculous individuals and tuberculous cattle. When contracted by persons from a human source the bacilli should have the human type; when contracted from cattle we should find the bovine type common in the lesions of young children, less common in those of older children, and very rare in adults. The occurrence of the bovine type, or of transition forms, in the lesions of adults would signify an exception to the rule of infection through latent bacilli introduced into the body during childhood.

This conception of tuberculosis, like most other modern views of the disease, brings with it no encouragement to regard tuberculous dairy products with complacency; on the contrary, it stamps the tuberculous cow as one of the greatest dangers to which public health is exposed.

(10) The inhalation theory to account for the occurrence of pulmonary tuberculosis has been shown to be no longer tenable, because no substance can be carried into the finer bronchioles by the respiratory process, and because tuberculous lesions in the lung have been shown to spread from the vascular system, the finer capillaries, and not from the air passages. Dried and pulverized tuberculous material has been shown to lack infectiousness, and the infectious spray discharged from the mouths of tuberculous persons during speaking and coughing has been shown to be of importance only in their immediate environment, unless such persons are permitted to handle articles of food, to which the larger droplets of the spray may adhere. The introduction of bacilli into the body through the uninjured wall of the digestive tract, anywhere from the mouth downwards, has been shown to be the chief mode of infection with tuberculosis.

Consequently, fresh, virulent tubercle bacilli in articles of food must be regarded as the greatest of tuberculous dangers, and among these tubercle bacilli in butter, because of their frequent occurrence

and their long-continued life and virulence, must rank very high as a danger of the utmost significance for public health.

(11) It is imperatively necessary for the protection of public health that all dairy herds should be cleaned of tuberculous animals. It makes no difference whether the milk obtained from a tuberculous herd, or a herd that contains one or more tuberculous animals, is sold as milk or cream or butter; in all forms it is equally objectionable and dangerous. It is not a question of the exposure of children alone, but also of adults; the former drink more milk, but the latter eat butter oftener and in larger quantities.

(12) Until we are certain that the milk delivered to us by dealers is obtained from healthy cows in every way protected from exposure to tuberculosis, we should not use it until it has been pasteurized or sterilized, and all cream that is not above suspicion should at least be pasteurized before it is used in the preparation of butter.

While we are not special advocates of the pasteurization or sterilization of dairy products, we recognize that the public is forced to resort to some such expedient for its protection, not only against tuberculosis, but also against numerous other infections. Thoroughly clean dairy products require no pasteurization. While unclean, pasteurized milk is fairly safe, unclean raw milk is to-day the most important cause tolerated by civilization for unnecessary disease, suffering, and death.

NOTE.—Since the foregoing circular was written several additional guinea pigs have died at the Experiment Station as the result of inoculation with tuberculous butter, as follows: One guinea pig inoculated with butter 113 days old died sixty-six days after inoculation; one inoculated with butter 113 days old died eighty-two days after inoculation, and one inoculated with butter 133 days old died one hundred and eighteen days after inoculation. The cause of death in each case was uncomplicated, generalized tuberculosis. Another guinea pig inoculated with butter 133 days old is now sick (March 19, 1908) and will probably die in a few days. This proves conclusively that tubercle bacilli may live and retain their virulence in ordinary salted butter practically four and one-half months or longer.

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